Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Ataxin-3 promotes testicular cancer cell proliferation by inhibiting anti-oncogene PTEN

Zhan Shi [a, b, 1], Jiaxin Chen [a, 1], Xiangmin Zhang [c, 1], Jian Chu [c, 1], Zhitao Han [d], Da Xu [a], Sishun Gan [a], Xiuwu Pan [a], Jianqing Ye [a, **], Xingang Cui [a, *]

[a] Department of Urology, The Third Affiliated Hospital of Second Military Medical University (Eastern Hepatobiliary Surgery Hospital), Shanghai, People's Republic of China
[b] Department of Urology, Taizhou First People's Hospital, Taizhou, People's Republic of China
[c] Department of Urology, The Gongli Hospital of Second Military Medical University, Shanghai, People's Republic of China
[d] School of Medicine and Life Sciences, Nanjing University of Chinese Medicine, Nanjing, Jiangsu, 210023, People's Republic of China

ARTICLE INFO

Article history:
Received 28 May 2018
Accepted 10 June 2018
Available online xxx

Keywords:
Testicular cancer
Ataxin-3
PTEN
Cell proliferation
AKT/mTOR signaling

ABSTRACT

Human Ataxin-3 protein was first identified as a transcript from patients with Machado-Joseph disease (MJD), also known as spinocerebellar ataxia type 3 (SCA3). Recent studies have demonstrated that Ataxin-3 is involved in gastric cancer and lung cancer. However, the role of Ataxin-3 in testicular cancer (TC) remains poorly understood. This study aims to explore the significance of Ataxin-3 expression in TC. Firstly, we investigated 53 paired TC and para-tumor tissues and found that Ataxin-3 was overexpressed in TC tissues, and this overexpression of Ataxin-3 was correlated with tumor stages. Functionally, Ataxin-3 overexpression promoted cell proliferation, and Ataxin-3 knockdown inhibited cell proliferation. In addition, up-regulation of Ataxin-3 inhibited the expression of PTEN and activated the AKT/mTOR pathway. Conversely, inhibition of Ataxin-3 suppressed the expression of *p*-AKT and *p*-mTOR, and increased the expression of p-4EBP1. These findings may provide a better understanding about the mechanism of TC and suggest that Ataxin-3 may be a potential prognostic biomarker and therapeutic target for TC.

1. Introduction

Testicular cancer (TC) is a common malignancy accounting for about 1–1.5% of all cancers in men [1,2]. About 52,549 new diagnosed TC cases and 9906 TC-related deaths occurr worldwide each year [2]. TC is classified as several types, including germ cell tumors (GCTs), sex cord-gonadal stromal tumors, and secondary testicular tumors. About 95% TC cases arise from germ cells to generate "GCTs", followed by gonadal stromal tumors, mixed GCT and secondary testicular tumors [3–5]. Although there has been a great decline in the mortality of TC over the last several decades, the prognosis of TC patients with platinum-resistant tumors remains poor due to limited therapeutic options [6,7]. Therefore, it is very important to identify new tumor targets to prevent the recurrence and metastasis of TC.

The ubiquitin proteasome system (UPS) is known to play an important role in cellular homeostasis, and the process of deubiquitination is closely related to the occurrence of many kinds of tumors [8]. Ataxin-3 is a deubiquitinating enzyme that interacts with poly-ubiquitin chains through its Josephin domain in the N-terminus and its ubiquitin interaction motifs in the C-terminus [9]. Human Ataxin-3 protein is encoded by the ATXN3 gene located on chromosome 14q21, and many reports consider an abnormal expansion of a polyQ sequence in ataxin-3 as the cause of Machado-Joseph disease (MJD), also known as spinocerebellar ataxia type 3 (SCA3). Ataxin-3 also promotes genome integrity by stabilizing Chk1 and MDC1 [10,11]. A recent study reported that the expression of Ataxin-3 was decreased in gastric cancer compared with that in noncancerous gastric tissue and correlated with the clinicopathologic features. Sacco et al. [12] found that Ataxin-3 restricted phosphatase and tensin homolog (PTEN) transcription, a classical anti-oncogene that antagonizes the PI3K/AKT/mTOR pathway,

* Corresponding author.
** Corresponding author.
E-mail addresses: dr_yejianqing@smmu.edu.cn (J. Ye), cuixingang@smmu.edu.cn (X. Cui).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbrc.2018.06.047
0006-291X/

suggesting that Ataxin-3 may provide an autonomous complementary therapeutic target in lung cancers. However, its expression and roles in TC remain unclear [13–16].

The purpose of the present study was to investigate the expression and roles of Ataxin-3 in TC, and explore the underlying mechanism and relationship between Ataxin-3 and TC cells. The results showed that Ataxin-3 promoted testicular cancer cell proliferation by inhibiting PTEN and indirectly activating the AKT/mTOR signaling pathway.

2. Materials and methods

2.1. Tissue specimens

From January 2012 to October 2017, 53 pairs of testicular cancer and para-tumor tissue specimens were obtained from orchiectomy patients from Changzheng Hospital of the Second Military Medical University (Shanghai, China). Patient data including age, histology, stage classification and metastatic sites are summarized in Supplement Table 1. The study was conducted with the understanding and written consent of each patient. The study protocol was approved by the Institutional Review Board of the said university. In addition, the histological differentiation and histological types of these specimens were evaluated by the experienced pathologists.

2.2. Immunohistochemical (IHC) and immunofloerescent (IFC) staining assays

IHC staining using an indirect immunoperoxidase technique, with the antibodies against ataxin-3 (ab96316, Abcam, USA) was performed using standard histological procedures described in the Histostain-Plus (DAB) kit (Mingrui Biotech) manual. The expression was recorded after evaluating the staining intensity of positive cells. Results were analyzed by standard light microscopy. IFC staining was performed with primary and secondary antibodies diluted in 10% bovine serum albumin (BSA) and the nuclei were stained by DAPI (Sigma). All fluorescent secondary antibodies were used at a dilution of 1:200 for 30 min (invitrogen). Quantification of IFC was performed with NIH Image J.

2.3. Western blot

Total protein was first extracted using SDS-PAGE and then transferred to a PVDF membrane (Thermo, USA). Afterwards, the PVDF membrane was incubated with 1:1000 dilution of antibody: ataxin-3(ab96316, Abcam, USA), AKT(ab8805, Abcam, USA), P-AKT(ab126433, Abcam, USA), mTOR(ab2732, Abcam, USA), P-mTOR(ab137133, Abcam, USA), 4EBP1(ab75767, Abcam, USA). The membrane was washed and incubated with a 1:2000 dilution of horseradish peroxidase-conjugated goat anti-rabbit (Santa Cruz, USA). SuperSignalMT West Puico PLUS (Termo Scientific, USA) was used to develop the blot, using β-actin (ab8226, Abcam, USA) as the loading control. All experiments were performed in triplicate.

2.4. Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted by using TRIZOL (Invitrogen, USA) and then reverse-transcribed into cDNA by using Prime ScriptTM RT Master Mix (Takara, Japan). Then, gene transcripts were quantified using the SYBR® Premix Ex TaqTM II (Takara, Japan) on the 7900HT Fast Real-Time PCR System (Life Technologies Corporation, USA) and normalized with GAPDH. Primer sequence: GAPDH-F: ACC AGG GCT GCT TTT AAC TC; GAPDH-R:TTG CCA TGG GTG GAA TCA TA; Ataxin-3-F: ATA AGC AAT GCC TTG AAA GT; Ataxin-3-R: AAT TTT CTA ACT GTA AAC CA.

2.5. Ataxin-3 overexpression plasmid and siRNA

*Hin*dIII and *Xho*I (Takara, Japan) was used to digest the pcDNA3.1 + plasmid. The coding sequence for ataxin-3 was synthesized by GENEWIZ and inserted into pcDNA3.1 + using a Quick-Fusion cloning kit (Biotool, USA). Two different siRNA oligonucleotides directed against ataxin-3 were purchased from Shanghai Genechem Co, Ltd (China). The siRNA1: GAUAUUUCCA-GAAGGCUGCUG; siRNA2: GUAAACCAGUGUUCCUUAUAA. Western blot analysis confirmed the expression of ataxin-3 after transfection.

2.6. Cell culture and transfection

Both TC cell lines (TCam-2 and I-10) and normal testis Hs1. Tes were purchased from the American Type Culture Collection (ATCC) and routinely maintained in RPMI-1640 (Gibco, USA) and DMEM (Gibco), and supplemented with 10% FBS (Gibco), 100 U/ml penicillin sodium and 100 mg/ml streptomycin. Cells were maintained in a humid atmosphere with 5% CO2 at 37 °C.

2.7. CCK8 assay

Cells were digested, counted, inoculated in 96-well plates, cultured for 24 h, and finally evaluated using the Cell Counting Kit 8 (Biotool, USA). The absorbance at 450 nm was measured using an ELx800 plate reader (BioTek Instruments Inc., USA).

2.8. Statistical analysis

All statistical analyses were performed by using SPSS 19.0 statistical software (SPSS Inc., Chicago, IL). All data are expressed as mean ± standard error of the mean (SEM). Assuming bilateral independent variance and Student *t*-test were used to evaluate the statistics of the mean values between groups. All experiments were repeated at least three times to obtain data and representative experiments were shown. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Ataxin-3 is expressed at a high level in TC

The expression of ataxin-3 in the 53 paired TC and para-tumor tissues was detected by IHC staining (Fig. 1A). The results showed that ataxin-3 was significantly up-regulated in TC tissues as compared with that in normal testicular tissues (Fig. 1A). In addition, the statistical results showed that the expression level of ataxin-3 was positively correlated with disease stage and metastasis (Supplement Table 1 and Table 2). Then, the expression of ataxin-3 protein and mRNA were detected by Western blot and real time-PCR in these cancer and para-tumor normal tissues (Fig. 1B and C). The result was consistent with the result of IHC staining ($p < 0.001$). These results showed that ataxin-3 was abnormally overexpressed in TC tissues.

3.2. Ataxin-3 overexpression promotes TC cell proliferation and Ataxin-3 knockdown inhibits TC cell proliferation

To detect the function of ataxin-3 in TC cells, the expression level of Ataxin-3 was first measured by Western blot in two TC cell lines and normal testicular cells (Fig. 2A). The overexpression plasmid Ataxin-3-pcDNA3.1 + was first constructed and then transfected in TCam-2 and I-10 cells. The effect of the expression was verified by Western blotting (Fig. 2B). When ataxin-3 protein was successfully

Please cite this article in press as: Z. Shi, et al., Ataxin-3 promotes testicular cancer cell proliferation by inhibiting anti-oncogene PTEN, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.06.047